UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: March 27, 2018

SUBJECT: Cerevisane (cell walls of *Saccharomyces cerevisiae* strain LAS117): Summary of Hazard and Science Policy Council (HASPOC) Meeting on February 8, 2018: Recommendations on the need for waiver for the 90-Day Dermal Toxicity Study (OSCPP 870.3250); and recommendations on the acceptance of data submitted from the literature for the following studies: 90-Day Oral Toxicity Study (OCSPP 870.3100), Prenatal Developmental Toxicity Study (OSCPP 870.3700) and Mutagenicity Testing Studies (OSCPP 870.5100, 870.5300, 870.5375).

PC Code: 100054

Decision No.: 524331; 524333; 524334
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0057714
MRID No.: N/A

DP Barcode: 437314; 437315; 437317
Registration No.: 91810-R; 91810-E
Regulatory Action: N/A
Case No.: N/A
CAS No.: N/A
40 CFR: N/A

FROM: Austin Wray, Executive Secretary
HASPOC
Health Effects Division (7509P)

THROUGH: Kelly Lowe, Co-Chair
Anwar Dunbar, Co-Chair
HASPOC
Health Effects Division (7509P)

TO: Shenell Bolden, M.S., Biologist
Judy Facey Ph.D. Toxicologist/ Acting Branch Chief
Risk Assessment Branch (RAB)
Biopesticides & Pollution Prevention Division (7511P)

MEETING ATTENDEES:

HASPOC Members: Kelly Lowe, Anwar Dunbar, Elissa Reaves, Elizabeth Mendez, John Kough, Jonathan Chen, Evisabel Craig, Michael Metzger, Ray Kent, Kristin Rickard, Chris Schlosser

Presenter: Shenell Bolden

Other Attendees: Austin Wray, Melinda Wilson, Jorge Muniz-Ortiz, Yung Yang, Tim McMahon, Judy Facy, Julie Van Alstine, Menyon Adams, Timothy Dole, Patricia Biggio

I. PURPOSE OF MEETING

BPPD's Risk Assessment Branch (RAB) is evaluating a request for registration of Cerevisane, a new biochemical Technical Grade Active Ingredient (TGAI) that is derived from the whole cell wall extract of *Saccharomyces cerevisiae* strain LAS 117. The whole cell wall extract from this non- genetically modified organism (non-GMO) strain of *Saccharomyces cerevisiae* yeast contains no viable cells, and is rich in carbohydrates. Cerevisane aids in the up-regulation of plant defense genes resulting in physiological changes including the reinforcement of plant cell walls and the production of antimicrobial compounds including hydrogen peroxide and phytoalexins (which are known to inhibit mycelium growth and the fruitification of susceptible fungal pathogens).

Cerevisane is obtained as an autolysis product from *Saccharomyces cerevisiae* strain LAS 117 cells. When these cells die, their enzymes retain activity and degrade cellular material, resulting in the release of small, soluble molecules into the bulk fluid. The cell walls (complete with the yeast cell membrane) are separated from these soluble molecules and used to formulate the final ingredient. Living cells of *Saccharomyces cerevisiae* strain LAS117 are not present in the end product, Cerevisane.

In order to fulfill the toxicological study data requirements, the applicant has submitted studies that have been published in the literature and rationale that includes the following: 1) studies of repeated oral exposure to yeast cell wall preparations from *Saccharomyces cerevisiae* are available in the published literature and can potentially be used risk assessment; 2) dried cell walls of *Saccharomyces cerevisiae* are approved by the U.S. Food and Drug Administration (USFDA) as a direct food additive; and 3) *Saccharomyces cerevisiae* has been used for centuries as a leavening agent in bread and an aid to fermentation.

The HASPOC met on February 8th, 2018 to discuss the data waiver for the 90-Day Dermal Toxicity Study (OSCPP 870.3250); and whether the submitted literature studies can be used the fulfill the data requirements for the 90-Day Oral Toxicity Study (OSCPP 870.3100); Prenatal Developmental Toxicity Study (OSCPP 870.3700) and Mutagenicity Testing Studies (OSCPP 870.5100, 870.5300, 870.5375).

II. SUMMARY OF USE PROFILE, EXPOSURE, AND HAZARD CONSIDERATIONS

a. Use and Exposure Profile

This is an application for a Section 3 Registration of a new active ingredient, Cerevisane, the whole cell walls extract of *Saccharomyces cerevisiae* strain LAS 117. The end product, ROMEO, is 94.1% ai and is to be applied as a foliar spray to field or greenhouse crops and is not to be applied through any type of chemigation. ROMEO is to be applied using conventional spray equipment to the point of saturation, with sufficient volume of mixture to ensure complete coverage of vegetation without run-off. It is recommended that medium to course spray be used in order to prevent drift. The application rate is 0.23 to 0.68 lbs. ai/A and the spray interval is 7-10 days depending on disease pressure. The following crops are included: Grapes, Cucurbits, Fruiting Vegetables, Root Vegetables, Bulb Vegetables, Brassica, Legume Vegetables, Herbs, Pome Fruits, Stone Fruits, Tree Nuts, and Tropical Fruits. PPE requirements includes the following; 1) waterproof gloves; 2) shoes and socks; 3) protective eyewear; and 4) long sleeve shirt and long pants.

Humans are already exposed to *Saccharomyces cerevisiae* due to its ability to ferment sugars into ethanol in the manufacturing of beers, wines, and liquor that are intended for human consumption. *Saccharomyces cerevisiae* has been used in the brewing industries since ancient times. Originally, it was isolated from grape skin. It is also called budding yeast or baker's yeast. Many human genes which are homologous to yeast genes have been identified with respect to cell division, cell signaling, protein processing, etc. The European Commission (2008) noted that US companies produced hundreds of thousands of metric tons of yeast for human consumption (bread and alcohol production) in 2012.

No dermal toxicological endpoints were identified from the toxicology database for any routes of occupational exposure. An acute dermal toxicity study was not conducted because the historically ubiquitous exposure of *Saccharomyces cerevisiae* to humans has not resulted in toxic effects. The rationale is based on the following: 1) baker's yeast is used to make most breads rise, so dermal exposure to the yeast occurs regularly without ill effects; 2) an acute oral toxicity study on a *Saccharomyces cerevisiae* cell wall preparation similar to Cerevisane Technical resulted in no adverse effects in rats following exposure to 2,000 mg/kg bw (Babíček, *et al*., 2007); and 3) the *Saccharomyces cerevisiae* cell wall preparation is composed of molecules that are insoluble and too large to penetrate the skin; consequently systemic exposure and toxicity are not expected. The Primary Dermal Irritation (OCSPP 870.2500; Richeux 2011a), and Dermal Sensitization (OCSPP 870.2600; Richeux 2011b) studies showed slight irritation and negative dermal sensitization, respectively. Sufficient data are available to support registration of Cerevisane Technical as a Toxicity Category IV for acute dermal exposure. There are currently no residential uses associated with this active ingredient. An aggregate risk assessment for ROMEO for dietary (food and drinking water) exposures was not conducted as no toxicological endpoints have been identified in the toxicity database. Furthermore, there is reasonable certainty that no harm will result to the general population or to infants and children from aggregated exposure to ROMEO (Cerevisane) based on the current use of *Saccharomyces cerevisiae* to humans as baker's yeast.

Table 1.0 Product Identity for Cerevisane Technical	
Product Name	Cerevisane Technical
Trade Name	Cerevisane Technical
Chemical name	Cerevisane (cell walls of *Saccharomyces cerevisiae* strain LAS117)
Common Names	Cell Walls of Baker's Yeast
CAS No	NA
Molecular Weight	NA
Chemical Formula	NA
Regulatory Status	Cerevisane (cell walls of *Saccharomyces cerevisiae* strain LAS117) is not presently registered with the U. S. EPA
Culture Depository	*Saccharomyces cerevisiae* strain LAS117 is deposited in the Collection Nationale de Cultures de Micro-organisms (CNCM) – Reference Number: LAS117 (Certificate CNCM, I-4512).

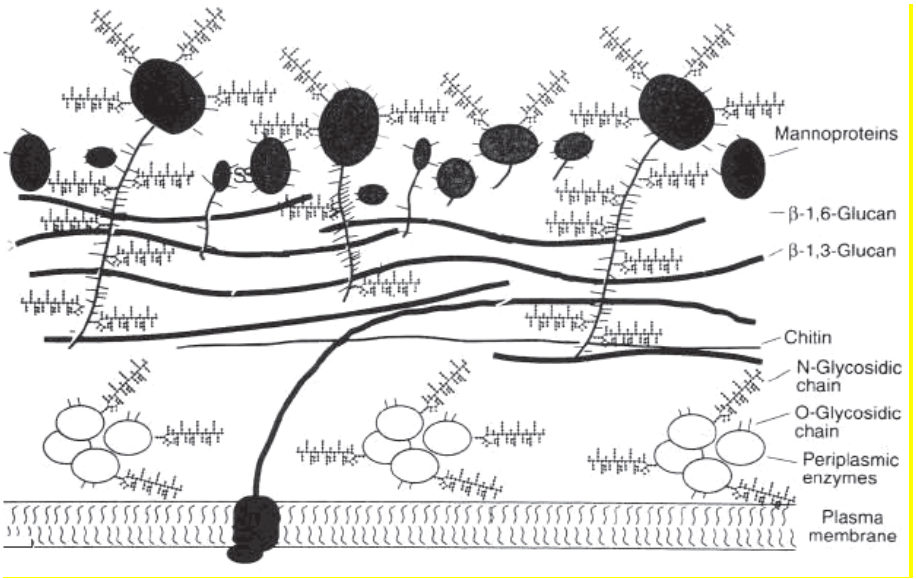

Figure 1.0 Composition and structure of the yeast cell wall.

b. Toxicity Profile

In order to fulfill the toxicological study data requirements, studies using *Saccharomyces cerevisiae* cell walls have been submitted from science literature studies. Using its literature guidance policy (USEPA 2012), EPA has evaluated each study for technical adherence to its test guidelines to determine each study's adequacy for use in human health risk assessment.

A study on acute oral toxicity (OCSPP 870.1100; Babíček, *et al.* 2007; MRID 50423801) concluded the acute oral LD_{50} was greater than 2,000 mg/kg. Acute primary eye irritation (870.2400; Richeux, F. 2011a; MRID 49682603) showed that there was initially moderate

redness, but complete recovery after 24 hrs. The primary dermal irritation (OCSPP 870.2500; Richeux, F. 2011b; MRID 49682604), and dermal sensitization (OCSPP 870.2600; Richeux, F. 2011c; MRID 49682605) showed slight irritation and negative dermal sensitization, respectively.

Subchronic studies showed that *Saccharomyces cerevisiae* has no effect on test organisms. A 90-day oral toxicity in the rat (OCSPP 870.3100; Babíček, *et al.* 2007; MRID 50423801) was conducted and showed a NOAEL of 100 mg/kg bw/day (HDT). A prenatal developmental toxicity study in pigs (OCSPP 870.3700; Graugnard *et al.,* 2014; MRID 50423806) showed that at 900 mg/kg/day (HDT) administered by diet, there was no effect on growth, teratology or mutagenic response. Bacterial reverse mutation (OCSPP 870.5100; Kogan, *et al.,* 2008; MRID 50423807) also proved that *Saccharomyces cerevisiae* cell walls were not mutagenic and an *in vitro* mammalian chromosome aberration study (OCSPP 870.5375; Krizkova *et al.,* 2001; MRID 50423807) showed anti-mutagenic and anti-clastogenic effects.

III. LITERATURE STUDIES SUBMITTED TO FULFILL THE DATA REQUIREMENTS

a. 90-Day Oral Toxicity (OCSPP 870.3100) (MRID 50423801; Babíček *et al.,* 2007)

A 90-day oral toxicity study in rats was conducted using *Saccharomyces cerevisiae* yeast cell wall-derived from 1,3-β-D-glucan as the test material (Babíček *et al.,* 2007). This 1,3-β-D-glucan yeast cell wall fraction contains some protein, fat, ash and moisture, similar to the Cerevisane Technical cell wall preparation (Babíček *et al.,* 2007). Also, similar to Cerevisane Technical, this 1,3-β-D-glucan preparation appears as a shell of the yeast cell wall, but in this case, much of the mannoprotein has been removed, while mannoprotein remains in Cerevisane Technical. Groups of 20 rats (10 males and 10 females) were randomly assigned to one of four groups. The groups received 0, 2, 33.3, or 100 mg/kg body weight/day of the β-glucan biopolymer via gavage as a suspension in sterile water for 91 consecutive dosing days. Animals received detailed examinations prior to initial dosing and weekly thereafter. Functional observational evaluations were performed in addition to clinical pathology and histopathology. No significant differences in food consumption or body weight were observed between any of the treatment groups during the study in males or females. Sporadic significant differences in organ weights were reported, but none were associated with pathologic processes and absolute weights were within historical control ranges. There were no increases in the incidence of histopathological findings observed in the group treated with the highest dose of test material. The study authors concluded that the No-Observed-Adverse-Effect-Level (NOAEL) was the high dose tested of 100 mg/kg bw/day (Babíček *et al.,* 2007).

The HASPOC recommends that the 90-day oral study data requirement be fulfilled using the submitted literature study.

b. Prenatal Development (OCSPP 870.3700) (MRID 50423804; Graugnard *et al*., 2014)

The requirement for a prenatal development study for Cerevisane Technical is satisfied based on the following effects to piglets from the administration of "yeast cell walls containing a mannan-

rich fraction" (MRF) that was given to sows during pregnancy and lactation (Graugnard *et al*., 2014). Two-hundred eighteen pregnant sows were assigned to one of two groups to receive either a basal diet or a basal diet supplemented with 900 mg/kg of MRF. From gestation Days 3 through Day 100, sows were fed to maintain body condition, and from day 100 until parturition, each received 0.9 kg extra feed. After parturition sows were fed *ad libitum*. There were no differences in the number of piglets born alive or dead between sows fed the control diet and the MRF-supplemented diet, and no differences in litter birth weights between the control and treated groups. Milk samples were collected from individual sows during lactation weeks 1 to 4. Ten days after parturition, one piglet per sow was sacrificed and the digestive tract was dissected and the jejunum flash frozen and processed for gene expression analysis. There were no differences in the number of piglets born alive or dead between sows fed the control diet and the MRF-supplemented diet, and no differences in litter birth weights between the control and treated groups (Graugnard *et al*., 2014). In addition, the number of piglets weaned per sow, the adjusted weaning weight, the adjusted weight gains and the pre-weaning mortality did not differ between treatments. The milk samples from sows showed that most components did not differ between treatments, but protein and total solids less fat ($P = 0.01$ and $P = 0.03$, respectively) were significantly greater in milk from the sows fed MRF compared with the controls. Milk IgA and IgM concentrations were similar in the two treatment groups, but IgG concentration was significantly greater in the MRF-supplemented group ($P = 0.03$). This increase in IgG in colostrum of sow's that received the yeast cell wall supplement is believed to enhance piglet performance by improving immunocompetence during a fundamental early stage of development. No adverse effects on the sow or offspring were reported from administration of a yeast cell wall preparation to sows from gestation day 3 through lactation. No adverse effects on the sow or offspring were reported from administration of a yeast cell wall preparation to sows from gestation day 3 through lactation. These data as well as the already widespread exposure of women to *Saccharomyces cerevisiae* confirm that no prenatal effects would be expected.

The HASPOC recommends that the prenatal development study data requirement be fulfilled using the submitted literature study.

c. Bacterial Reverse Mutation Test (OCSPP 870.5100) (MRID 50423807; Miadoková *et al*., 2005)

Existing information performed on a glucan component of a yeast cell wall preparation from *Saccharomyces cerevisiae* provides a reasonable surrogate for Cerevisane Technical (Miadoková *et al*., 2005) to fulfill the data requirement for the Bacterial Reverse Mutation Test study. This assay was performed to see if the glucan could reverse the mutagenic effect of 9-aminoacridine (9-AA), methyl methanesulfonate (MMS) Maleic hydrazide or Ofloxacin. Salmonella typhimurium tester strains TA97, TA98, TA100 and TA102 were used. Three concentrations of glucan (750, 500, or 250 µg/plate) were placed on minimal agar plates either alone or with the appropriate diagnostic mutagen. The plates were incubated at 37° C and His+ revertants were counted after 72 hours. The glucan did not increase revertants above the negative control in the tester strains, and inhibited 9AA significantly only at the high concentration in the TA97 tester strain.

The HASPOC recommends that the bacterial reverse mutation test data requirement be fulfilled using the submitted literature study.

d. *In vitro M*ammalian Cell Assay (OCSPP 870.5300, 5375) (MRID 50423807; Miadoková *et al*., 2005)

Saccharomyces cerevisiae cell wall extracts are generally considered to have anti-oxidant and anti-mutagenic effects rather than mutagenic effects (US FDA 2007; Biothera Inc. 2008. GRAS Consensus Statement). One article set out to characterize the potential anti-genotoxic effects of carboxymethyl glucan derived from a *Saccharomyces cerevisiae* cell wall preparation (Miadoková *et al.,* 2005). In an assay of a recombination-repair deficient strain of a unicellular green alga, *uvs*10, the glucan significantly reduced cytotoxicity and mutagenicity produced by the diagnostic mutagen, methyl methanesulfonate (MMS). Another assay evaluated the potential phytotoxicity and anti-clastogenicity of the glucan in the plant species *Vicia sativa*. The glucan significantly reduced the clastogenic effect produced by the diagnostic clastogenic, maleic hydrazide. The authors suggest the anti-mutagenic and anti-clastogenic effects of the glucan are due to the antioxidant properties that allow it to scavenge reactive oxygen species.

The HASPOC recommends that the *in vitro* mammalian cell assay requirement be fulfilled using the submitted literature study.

IV. WAIVER REQUEST

a. 90-Day Dermal Toxicity (OCSPP Guideline: 870.3250)

The 90- day dermal study is a conditional data requirement for biopesticides with a food use registration. A waiver for a 90-Day Dermal Toxicity study for Cerevisane Technical was requested based on the following: 1) subchronic oral toxicity studies on *Saccharomyces cerevisiae* cell wall preparations similar to Cerevisane Technical resulted in no adverse effects at the highest dose tested (100 mg/kg bw); 2) the dried cell wall preparation of *Saccharomyces cerevisiae* is composed of the yeast cytoskeleton and internal membranes that are too large to penetrate skin and cause systemic effects, and; 3) baker's yeast is used to make most breads rise, so a great percentage of the population experience exposure to the live yeast without ill effects.

Therefore, based on a WOE approach, considering all available Cerevisane hazard and exposure data, the HASPOC recommends that a 90-Day Dermal study is not required at this time. This approach includes the following considerations: 1) historically ubiquitous exposure of *Saccharomyces cerevisiae* to humans has not resulted in toxic effects; 2) an oral toxicity study on a *Saccharomyces cerevisiae* cell wall preparation similar to Cerevisane Technical resulted in no adverse effects in rats following acute exposure to 2,000 mg/kg bw and subchronic exposure up to 100 mg/kg bw/day (Babíček, *et al*., 2007); and 3) the dried cell wall preparation of *Saccharomyces cerevisiae* is composed of the yeast cytoskeleton and internal membranes that are too large to penetrate skin and cause systemic effects.

V. HASPOC CONCLUSIONS

Based on a WOE approach, considering all the available hazard and exposure data for Cerevisane, the HASPOC concludes that the 90-day dermal toxicity study **is not required** at this time.

In addition, based on the accepted studies submitted from the literature, the HASPOC concludes that the data requirements for the 90-day oral toxicity (OCSPP 870.3100), prenatal development toxicity (OCSPP 870.3700), and mutagenicity toxicity (OCSPP 870.5100) have been fulfilled.

VI. REFERNCES

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